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The effect of chloramphenicol, actinomycin *D* and 5-bromouracil on the synthesis of photosynthetic pigments*

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Abstract

The present study concerned the effect of chloramphenicol (100 µg/ml), actinomycin *D* (30 µg/ml), and 5-bromouracil (190 µg/ml) on the accumulation of chlorophyll *a*, chlorophyll *b*, β-carotene and four fractions of xanthophylls (with the domination of: lutein, zeaxanthin, violaxanthin and neoxanthin) in the primary bean leaves. The pigment content was determined in etiolated leaves after exposure to light for different lengths of time.

It results from this study that chloramphenicol inhibits β-carotene synthesis more than do other pigments. The formation of xanthophylls and chlorophyll *b* is relatively less sensitive to the action of this antibiotic. Actinomycin *D* is also a somewhat more effective inhibitor of the accumulation of β-carotene than other pigments. In 5-bromouracil-treated leaves the accumulation of all carotenoids is inhibited almost to the same extent.

These results suggest that the accumulation of chlorophyll *b* and xanthophylls is a little less dependent upon the activity of 70 S ribosomes in chloroplasts than the accumulation of chlorophyll *a* and β-carotene.

INTRODUCTION

Chloramphenicol, actinomycin *D*, and 5-bromouracil, well known inhibitors of protein or nucleic acid biosynthesis, are also often used for studies of some aspects of photosynthetic apparatus formation (for review see Kirk and Tilney-Bassett, 1967). In the presence of these inhibitors the accumulation of chlorophylls and carotenoids becomes partially inhibited (Margulies, 1962, Gassman and Bogorad, 1967, and others). The modes of action of these inhibitors are still far from clear. However, many results obtained with both bacterial and *E. gracilis* cells as well

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as with higher plants have suggested that only 70 S ribosomes are sensitive sites for chloramphenicol action (Stutz and Noll, 1967, Smillie *et al.*, 1967, Eisenstadt and Brawermann, 1964, Ellis, 1969). Actinomycin *D* induces the inhibition of the DNA-directed synthesis of RNA molecules presumably by chloroplasts as well as the nucleus (Kirk, 1964, Matsushita and Mori, 1972). Furthermore, 5-bromouracil (similarly to 5-fluorouracil) is a purine antagonist (see Kirk and Tilney-Bassett, 1967). In the presence of 5-fluorouracil or 5-fluorodeoxyuridine also the synthesis of thymidylic acid seems to be blocked, at least in bacterial cells (Cohen *et al.*, 1958, Van Noort and Wallace, 1963).

Moreover, recent evidence suggests that the reduction of the pigment accumulation in the inhibitor-treated leaves might be due to the inhibition of biosynthesis of lamellar structural protein and a number of enzymes involved in the pigment biosynthesis (see Kirk and Tilney-Bassett, 1967). There is a suggestion (Gassman and Bogorad, 1967) that the inhibition of chlorophyll accumulation in the presence of chloramphenicol is due to instability of δ -aminolaevulinic acid synthetase. Supposedly this enzyme undergoes rapid turnover and disappears from the cells as soon as its biosynthesis is stopped.

Relatively few reports appear in the literature on the effect of these inhibitors on the carotenoid formation. For example, Kirk (1968) showed that in the case *E. gracilis* actidione and chloramphenicol inhibit accumulation of total carotenoids by about half as much as the accumulation of chlorophyll. Similar results have been obtained with leaves of *Vicia faba* (Nikolayeva *et al.*, 1969) and wheat (Hofmann and Walter, 1970) treated with chloramphenicol.

This paper is concerned with the comparison of the effects of chloramphenicol, actinomycin *D*, and 5-bromouracil on the biosynthesis of chlorophyll pigments with those of carotenoid pigments in etiolated primary bean leaves exposed to light.

MATERIAL AND METHODS

The experiments were carried out with the primary leaves of bean (*Phaseolus vulgaris* var. Krakowska) seedlings which had been cultivated in darkness for eight days, at constant temperature (24–25°C) and on liquid Hoagland's solution (for details see Więckowski, 1959, 1971).

Treatment with inhibitors. Before illumination of dark-grown seedlings chloramphenicol (D(-)-treo-chloramphenicol, pharm., Polfa, Poland), actinomycin *D* (Serva) or 5-bromouracil (Reagal, Hungary) were supplied to the leaves through roots. After incubation in darkness the seedlings being still in contact with the solution of inhibitor were exposed to the light of approximately 24 W m⁻² intensity of the photosynthetically active radiation.

In the preliminary experiments the inhibitors were supplied to the plants also either directly into the detached leaves by infiltration or by excised stem. These modifications influenced only the quantity and no quality of the pigment contents and therefore were eliminated from further experiments.

Pigment determination. Chlorophyll contents were estimated in 80% (v/v) acetone (p.a.) by spectrophotometry according to the technique of Mackinney (see Holden, 1965). After chlorophyll estimation the pigments were transferred into ether (pharm.) and then were saponified (Więckowski, 1961). Carotenoids were separated by thin layer chromatography according to the technique by Hager and Meyer-Bartenrath (1966). The adsorbent consisted of: Kieselgur (Merck), Kieselgel GF 254 (Merck), CaCO_3 and Ca(OH)_2 mixtured by ratio 12 : 3 : 3 : 0.02. The chromatogram was developed by the mixture of benzine (95°—125°C), acetone and chloroform (50 : 25 : 20 by volume). This procedure made it possible to obtain five coloured layers on the developed chromatogram with successive predominance of (from the front): β -carotene, lutein, zeaxanthin, violaxanthin, and neoxanthin. The separated pigments were eluted from the adsorbent by chloroform (β -carotene) or ethanol (xanthophylls). The optical densities were assayed by the Hilger Uvispek spectrophotometer at the main absorption peak of individual pigments and then the absolute values of pigment concentrations were calculated (Davies, 1965).

To avoid photooxidation of pigments the whole procedure was carried out in dim light and, if possible, in N_2 atmosphere.

The identification of separated pigments was performed by the analysis of Rf values and absorption spectra in petrol ether, ethanol and chloroform. It appeared by rechromatography that fractions separated in this way were not quite pure; beside the pigments mentioned above there occurred small contaminations by some isomers in most of these fractions.

All experiments were repeated 3 to 5 times. The data on the diagrams represent mean values. In most cases calculated standard deviation does not excess 10 per cent of mean value of the individual pigment content. Only in a very few experiments its value for some xanthophyll content reached approximately 20 per cent.

RESULTS

Chloramphenicol applied in the concentration of 100 $\mu\text{g/ml}$ did not inhibit the biosynthesis of different pigments to the same extent (Fig. 1). In the presence of this antibiotic the accumulation of β -carotene is most inhibited in the etiolated seedlings exposed to the light for 72 hours; in comparison with the control, only about 6 per cent of β -carotene is formed in chloramphenicol-treated leaves. Under the same conditions the accumulation of xanthophylls was about 10-fold as high as that of β -carotene. Chloramphenicol is only a little more effective in the inhibition of chlorophyll than xanthophyll synthesis. The synthesis of chlorophyll *b* is a little less reduced than that of chlorophyll *a*.

The inhibitory effect of this drug on greening may be observed well already after 24 hours of illumination of etiolated seedlings. In comparison with the control the biosynthesis of pigments does not fall off very sharply at that time: the biosynthesis of β -carotene is prevented by about 70 per cent, and about 40 per cent inhibition was caused in the biosynthesis of xanthophylls and chlorophylls.

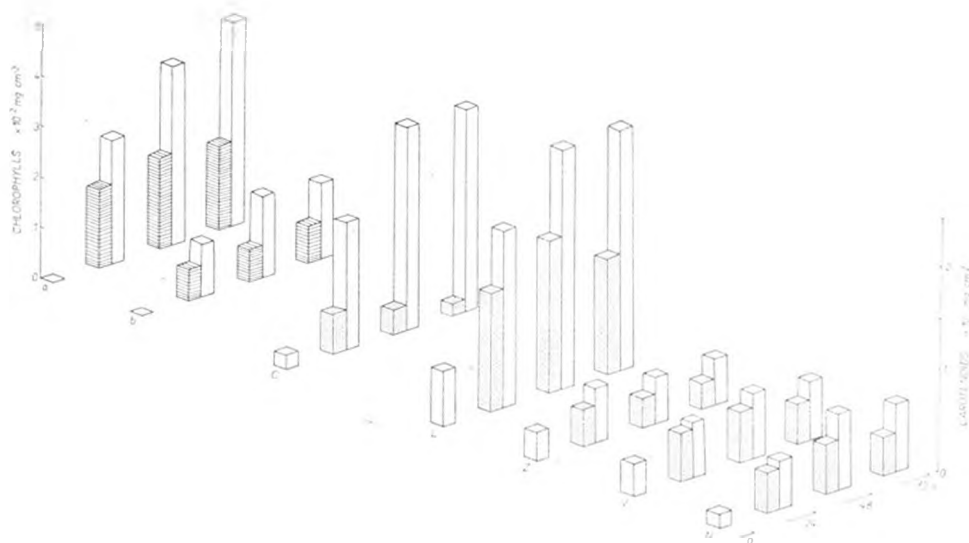


Fig. 1. Effect of chloramphenicol (100 $\mu\text{g/ml}$) on the accumulation of photosynthetic pigments in etiolated leaves exposed to light. Before illumination the seedlings were incubated with inhibitor in the dark for two hours. Shaded columns — treated with inhibitor, non-shaded columns — control a — chlorophyll a, b — chlorophyll b, c — β -carotene, l — lutein, z — zeaxanthin, v — violaxanthin, n — neoxanthin.

A progressive diminution in the concentrations of some carotenoid fractions, especially those containing β -carotene, lutein and zeaxanthin, was observed in the chloramphenicol-treated leaves during illumination, e.g. the content of β -carotene fell off to about one-third of that value recorded after 24 hours of illumination.

Actinomycin D was supplied to the seedlings at the concentration of 30 $\mu\text{g/ml}$. These experiments, however, should be limited to the 24 hours period of irradiation since after that time the treated leaves underwent necrosis. In 24 hours illuminated leaves the accumulation of xanthophylls and β -carotene reached about 60 per cent and about 30 per cent, respectively, of the control (Fig. 2). The accumulation of chlorophyll b seems to be a little more reduced than that of chlorophyll a: about 10 per cent less chlorophyll b than chlorophyll a was accumulated in actinomycin D-treated leaves.

The biosynthesis of all pigments was substantially high during the initial 12 hours of illumination of seedlings in the presence of the antibiotic and between 12 and 24 hours of illumination the accumulation of β -carotene was even abolished, whereas the accumulation of xanthophylls and chlorophylls occurred although to a limited extent.

The biosynthesis of chlorophylls in 5-bromouracil-treated leaves is also more inhibited than the biosynthesis of carotenoids (Fig. 3). In comparison with control, about 55 and 30 per cent of chlorophyll was accumulated by the inhibitor-treated leaves after 24 and 48 hours of illumination, respectively. Under the same circumstances, the accumulation of carotenoids was only slightly affected by this inhibitor. In this case, there was no preferential inhibition of β -carotene accumulation.

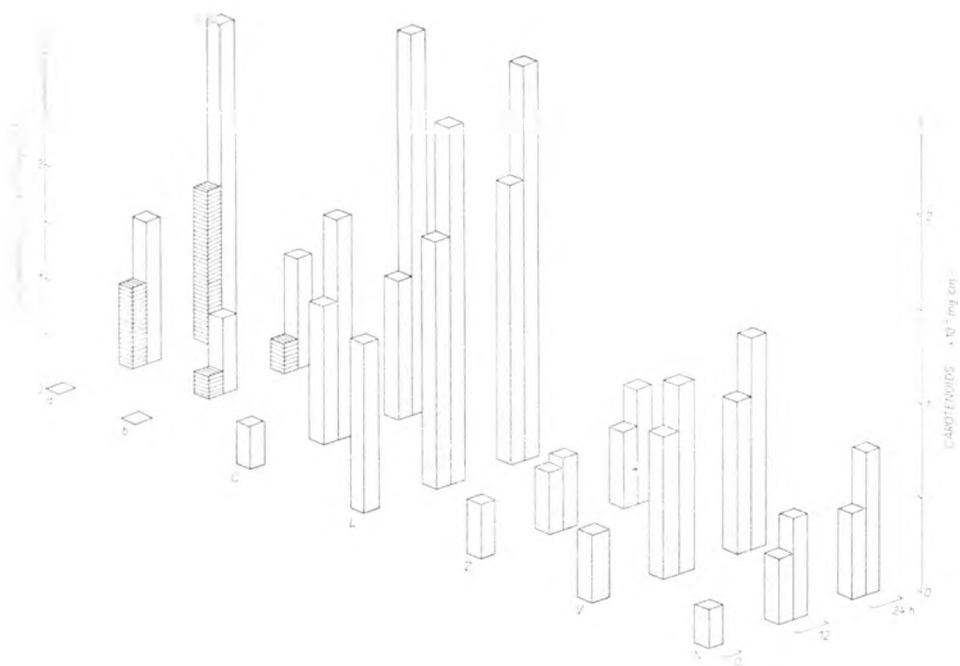


Fig. 2. Effect of actinomycin *D* (30 $\mu\text{g/ml}$) on the accumulation of photosynthetic pigments in etiolated leaves exposed to light. Other details as in Fig. 1.

DISCUSSION

Satisfactory explanation of the above presented results is difficult particularly since the effects of chloramphenicol, actinomycin *D*, and 5-bromouracil are not confined to the chloroplast metabolism but also interfere with many metabolic processes in other cell compartments. For example, it is known that chloramphenicol modified ion uptake, active transport, oxidative phosphorylation (Parthier, 1965, Ellis and McDonald, 1970, Ireland and Bradbeer, 1971). Therefore a decrease in the accumulation of only certain pigments in the leaves in the presence of any inhibitor might be a consequence of the cessation of protein or nucleic acid synthesis as well as a result of the modification of other metabolic pathways.

On the other hand, all three compounds used in the described experiments caused only partial inhibition of protein synthesis (see Margulies, 1964, Nikolayeva *et al.*, 1969, Kirk and Tilney-Bassett, 1967, and others), and until now it is not known if the biosynthesis of all kinds of protein in, for example, chloramphenicol-treated chloroplasts is inhibited to the same extent. In the interpretation of the results all these secondary effects of the applied inhibitors on the chloroplasts metabolism were tendentially neglected.

The present experiments showed a varying degree of individual pigment synthesis inhibition by chloramphenicol (100 $\mu\text{g/ml}$), actinomycin *D* (30 $\mu\text{g/ml}$), and 5-bro-

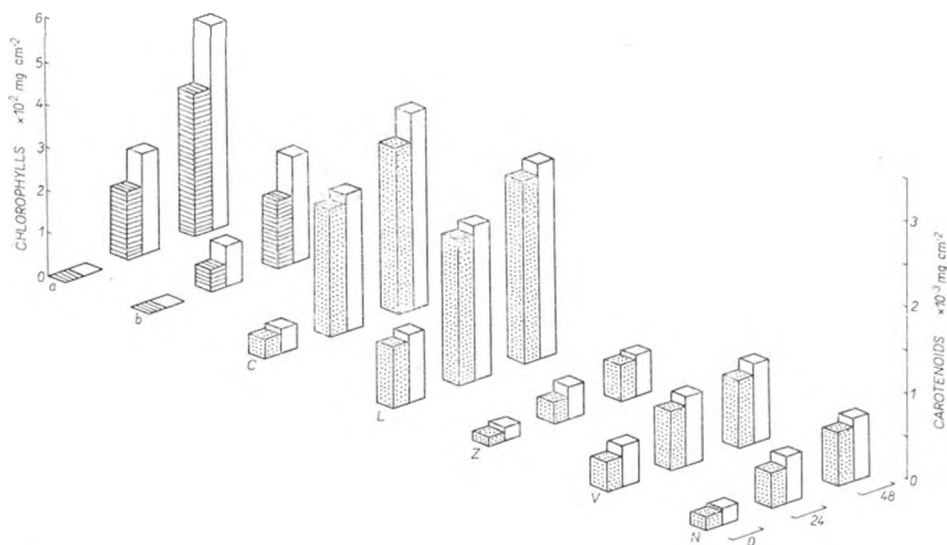


Fig. 3. Effect of 5-bromouracil (190 µg/ml) on the accumulation of photosynthetic pigments in etiolated leaves exposed to light. Other details as in Fig. 1.

mouracil (190 µg/ml) in etiolated leaves exposed to light. Chloramphenicol has a more immediate inhibitory effect on the β -carotene accumulation than on xanthophylls. Since this drug inhibits the protein synthesis on 70 S ribosomes it would seem that certain enzymes necessary for biosynthesis of β -carotene are synthesized within the chloroplast and the formation of xanthophylls is to a less extent dependent upon the biosynthesis of protein within this organelle. As has been shown by numerous authors (e.g. Yamamoto *et al.*, 1962, Goodwin, 1965), xanthophylls are formed by oxidation of suitable carotene pigments or their immediate precursors. It may be concluded that the proteins produced on the 70 S ribosomes are less essential for the conversion of carotenes into xanthophylls. Supposedly the accumulation of xanthophyll pigments is to a larger extent dependent upon the biosynthesis of structural protein of the lamellar system.

After 72 hours of illumination of leaves treated with chloramphenicol the biosynthesis of chlorophyll *a* is inhibited to a slightly higher degree than the biosynthesis of chlorophyll *b*. This result is in agreement with those of Banerji and Loloraya (1967), and Nikolayeva *et al.*, (1969). The degree of inhibition of chlorophyll *b* synthesis is approximately the same as inhibition of xanthophyll synthesis. After a shorter time of illumination the differences between the ratios of chlorophyll *a* to *b* in chloramphenicol-treated leaves and control are not significant, presumably since at that period there is some protein excess which has been formed before the treatment of seedlings with antibiotic.

It is commonly known that carotenoids and chlorophyll *b* are connected mainly with photosystem II and therefore the biosynthesis of protein of this photosystem is probably to a less extent related to the activity of the 70 S ribosomes within the

chloroplasts. This kind of interpretation is supported by the finding of Machold and Aurich (1972). These authors have given some data according to which the proteins of photosystem II are synthesized mainly on the 80 S ribosomes, and the proteins of photosystem I on the 70 S ribosomes within the chloroplasts.

The biosynthesis of xanthophylls is also slightly inhibited than that of carotenes in the leaves treated with actinomycin *D* in the concentration of 30 µg/ml, at least during the first 24 hours of illumination of dark-grown seedlings. It should mean that the transformation of carotenes into xanthophylls is less dependent upon the continuous synthesis of the essential RNA molecules than the synthesis of carotene itself. Similarly as in the case of chlorophyll formation it might be rRNA (Matsushita and Mori, 1972) or a specific mRNA (Kirk and Tilney-Bassett, 1967). It may perhaps be that RNA responsible for the synthesis of protein involved in the xanthophyll formation has a long life time and therefore the production of these pigments might occur due to RNA excess which has been formed before the antibiotic is supplied to the leaves.

In the presence of 5-bromouracil the inhibition of chlorophyll synthesis was also considerably greater than the carotenoid synthesis. In this case no preferential repression of β -carotene production occurred; the accumulation of β -carotene was inhibited to about the same extent as the accumulation of xanthophylls. It means that the synthesis of protein engaged in the xanthophyll formation is relatively more sensitive to the action of 5-bromouracil than that of actinomycin *D* or chloramphenicol. At present any interpretation of this phenomenon will have only speculative character. As was mentioned in the introduction it has been recognized that uracil analogs act as uracil antagonists in nucleic acid synthesis (see also Mandel, 1969). It is also known that 5-fluorouracil influence the ribosome formation (Aronson, 1961, Smillie *et al.*, 1963). Thus the inhibition of specific RNA synthesis as well as that of ribosomes activity may contribute to the preferential inhibition of protein synthesis essential for xanthophyll accumulation.

REFERENCES

- Aronson A. I., 1961. The effect of 5-fluorouracil on bacterial protein and ribonucleic acid synthesis. *Biochim. Biophys. Acta* 49: 98—197.
- Banerji D. and Laloraya M. M., 1967. Chlorophyll formation in isolated pumpkin cotyledons in the presence of kinetin and chloramfenikol, *Plant and Cell Physiol.* 8: 263—268.
- Cohen S.S., Flaks J. G., Barner H. D., Loeb M. R. and Lichtenstein J., 1958, The mode of action of 5-fluorouracil and its derivatives. *Proc. Natl. Acad. Sci.* 44: 1004—1012.
- Davies B. H., 1965. Analysis of carotenoid pigments, [in:] T. W. Goodwin, ed. *Chemistry and Biochemistry of Plant Pigments*, Academic Press, London and New York, pp. 489—532.
- Eisenstadt J., and Brawerman G., 1964, The protein-synthesizing systems from the cytoplasm and the chloroplasts of *Euglena gracilis*, *J. Mol. Biol.* 10: 392—402.
- Ellis R. J., 1969. Chloroplast ribosomes: stereospecificity of inhibition by chloramphenicol, *Science* 163: 477—478.
- Ellis R. J. and MacDonald I. R., 1970. Specificity of cycloheximide in higher plant systems. *Plant Physiol.* 46: 227—232.

- Gassman M. and Bogorad L., 1967. Control of chlorophyll production in rapidly greening bean leaves. *Plant Physiol.* 42: 774—780.
- Goodwin T. W., 1965. The biosynthesis of carotenoids, [In:] T. W. Goodwin, ed., *Chemistry and Biochemistry of Plant Pigments*, Academic Press, London and New York, pp. 143—173.
- Hager A. and Meyer-Bartenrath T., 1966. Die Isolierung und Quantitative Bestimmung der Carotinoide und Chlorophylle von Blättern, Algen und isolierten Chloroplasten mit Hilfe Dünnschichtchromatografischer Methoden. *Planta (Berl.)* 69: 198—217.
- Hoffmann P. und Walter G., 1970. Der Einfluss von Chloramphenicol und Streptomycin auf die Entwicklung des photosynthetischen Apparates bei Weizenkeimpflanzen, *Biol. Zbl.* 89: 163—200.
- Holden M., 1965. Chlorophylls, [In:] T. W. Goodwin, ed, *Chemistry and Biochemistry of Plant Pigments*, Academic Press, London and New York, pp. 461—488.
- Ireland H. M. M. and Bradbeer J. W., 1971. Plastid development in primary leaves of *Phaseolus vulgaris*, *Planta (Ber.)* 96: 254—261.
- Kirk J. T. O., 1964. DNA-dependent RNA synthesis in chloroplast preparations, *Biochem. Biophys. Res. Commun.* 14: 393—397.
- Kirk J. T. O., 1968. Studies on the dependence of chlorophyll synthesis on protein synthesis in *Euglena gracilis* together with a nomogram for determination of chlorophyll concentration, *Planta (Berl.)* 78: 200—207.
- Kirk J. T. O. and Tilney-Bassett R. A. E., 1967. The plastids, their chemistry, structure, growth and inheritance, W. H. Freeman and Company, London and San Francisco.
- Machold O. and Aurich O., 1972. Sites of synthesis of chloroplast lamellar protein in *Vicia faba*. *Biochim. Biophys. Acta*, 281: 103—112.
- Mandel H. G., 1969. The incorporation of 5-fluorouracil into RNA and its molecular consequences, [In:] F. H. Hahn, ed. *Progress in molecular and subcellular biology*, Springer-Verlag, New York, Heidelberg, Berlin, vol. 1, pp. 82—135.
- Margulies M. M., 1962. Effect of chloramphenicol on light dependent development of seedlings of *Phaseolus vulgaris* var. Black Valentine with particular reference to development of photosynthetic activity. *Plant Physiol.* 37: 473—480.
- Margulies M. M., 1964. Effect of chloramphenicol on light-dependent synthesis of proteins and enzymes of leaves and chloroplasts of *Phaseolus vulgaris*. *Plant Physiol.* 39: 579—585.
- Matsushita K. and Mori Y., 1972. The effects of actinomycin D on the greening of etiolated bean leaves. *Plant and Cell Physiol.* 13: 191—194.
- Nikolayeva M. K., Osipova O. P. and Nichiporovich A. A., 1969. Relation between protein metabolism and functional activity of the photosynthetic apparatus, [In:] H. Metzner, ed. *Progress in photosynthesis research*, Tübingen, vol. 1, pp. 409—418.
- Noort van D. and Wallace A., 1963. Inhibition of chlorophyll synthesis by 5-fluorouracil and 5-fluorodeoxyuridine, *Biochem. Biophys. Res. Comm.* 10: 109—111.
- Parthier B., 1955. Chloramphenicol-Wirkung auf eine durch Licht stimulierte Proteinsynthese in Blättern und isolierten Chloroplasten. *Z. Naturforsch.* 20b: 1191—1193.
- Smillie R. M., Evans W. R. and Lyman H., 1963. Metabolic events during the formation of a photosynthetic from a non-photosynthetic cell, *Brookhaven Symp. Biol.* 16: 89—197.
- Smillie R. M., Graham D., Dwyer M. R., Grieve A. and Tobin N. F., 1967. Evidence for the synthesis *in vivo* of proteins of the Calvin cycle and of the photosynthetic electron transfer pathway on chloroplast ribosomes. *Biochem. Biophys. Res. Comm.* 28: 604—610.
- Stutz E. and Noll H., 1967. Characterization of cytoplasmic and chloroplast polysomes in plants. Evidence for three classes of ribosomal RNA in nature. *Proc. Nat. Acad. Sci. USA* 57: 774—787.
- Więckowski S., 1959. The relation between the growth of leaves and the synthesis of chlorophyll, *Acta Biol. Crac. ser. Bot.* 2: 1—11.
- Więckowski S., 1961. Changes in the content of carotenoids in growing bean leaves. *Bull. Acad. Polon. Sc. ser. biol.* 9: 325—332.

- Więckowski S., 1971. Photosynthetic activity of bean leaves and the relative content of chlorophyll in particles isolated from chloroplasts after irradiation of etiolated seedlings, *Photosynthetica* 5: 44—49.
- Yamamoto H. Y. Nakayama T. O. and Chichester C. O., 1962. Studies on the light and dark interconversion of leaf xanthophylls, *Arch. Biochem. Biophys.* 93: 168—173.

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*I. Wpływ chloramfenikolu, aktynomycyny D i 5-bromouracylu na syntezę
barwników fotosyntetycznych*

Streszczenie

W pracy opisano wyniki badań nad wpływem chloramfenikolu (100 µg/ml) aktynomycyny D (30 µg/ml) i 5-bromouracylu (190 µg/ml) na akumulację chlorofilu *a*, chlorofilu *b*, β-karotenu i czterech frakcji barwników ksantofilowych (z przewagą: luteiny, zeaksantyny, wiolaksantyny i neoksantyny) w młodych liściach fasoli.

Przedstawione dane wskazują, że chloramfenikol hamuje w większym stopniu syntezę β-karotenu niż innych barwników. Powstawanie barwników ksantofilowych i chlorofilu *b* jest względnie mniej wrażliwe na działanie tego antybiotyku. Aktynomycyna D jest również nieco efektywniejszym inhibitorem nagromadzania się β-karotenu niż innych barwników. Akumulacja wszystkich karotenoidów w liściach traktowanych 5-bromouracylem jest hamowana mniej więcej w jednakowym stopniu.

Otrzymane wyniki sugerują, że synteza chlorofilu *b* i barwników ksantofilowych jest mniej uzależniona od aktywności rybosomów typu 70 S w chloroplastach niż synteza chlorofilu *a* i β-karotenu.